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PROSTATE-SPECIFIC POLYPEPTIDE PAMP AND ENCODING NUCLEIC ACID MOLECULES

BACKGROUND OF THE INVENTION

This invention relates generally to cancer and, more specifically, to a prostate-specific gene that can be used to diagnose and treat prostate cancer, including advanced or metastatic prostate cancer.

Cancer is currently the second leading cause of mortality in the United States. However, it is estimated that by the year 2000 cancer will surpass heart disease and become the leading cause of death in the United States. Prostate cancer is the most common non-cutaneous cancer in the United States and the second leading cause of male cancer mortality.

Cancerous tumors result when a cell escapes from its normal growth regulatory mechanisms and proliferates in an uncontrolled fashion. As a result of such uncontrolled proliferation, cancerous tumors usually invade neighboring tissues and spread by lymph or blood stream to create secondary or metastatic growths in other tissues. If untreated, cancerous tumors follow a fatal course. Prostate cancer, due to its slow growth profile, is an excellent candidate for early detection and therapeutic intervention.

During the last decade, most advances in prostate cancer research have focused on prostate specific antigen (PSA), a member of the serine protease family that exhibits a prostate-specific expression

profile. Serum PSA remains the most widely used tumor marker for monitoring prostate cancer, but its specificity is limited by a high frequency of falsely elevated values in men with benign prostatic hyperplasia (BPH). Other biomarkers of prostate cancer progression have proven to be of limited clinical use in recent surveys because they are not uniformly elevated in men with advanced prostate cancer. Due to the limitations of currently available biomarkers, the identification and characterization of prostate specific genes is essential to the development of more accurate diagnostic methods and therapeutic targets. In many cases, the clinical potential of novel tumor markers can be optimized by utilizing them in combination with other tumor markers in the development of diagnostic and treatment modalities.

Normal prostate tissue consists of three distinct non-stromal cell populations, luminal secretory cells, basal cells, and endocrine paracrine cells. Phenotypic similarities between normal luminal cells and prostate cancer cells, including the expression of PSA, have suggested that prostate adenocarcinomas derive from luminal cells. However, a number of recent studies suggest that at least some prostate cancers can arise from the transformation of basal cells and report the expression of various genes in normal prostate basal cells as well as in prostate carcinoma cells. These genes include prostate stem cell antigen (PSCA), c-met and Bcl-2. Because none of these genes is universally expressed in all basal cells and prostate carcinomas, the utility of these genes as diagnostic markers is limited. Likewise, because PSA is expressed in luminal secretory

cells in normal prostate tissue, this antigen has limited utility as a marker for basal cell derived carcinomas.

Thus, there exists a need for the identification of additional prostate specific genes that can be used as diagnostic markers and therapeutic targets for prostate cancer. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a PAMP nucleic acid molecule containing a nucleic acid sequence encoding substantially a PAMP polypeptide. A PAMP nucleic acid molecule of the invention encodes substantially the amino acid sequence shown as SEQ ID NO:2. A PAMP nucleic acid molecule can encode, for example, the amino acid sequence shown as SEQ ID NO:2 and, in one embodiment, contains the nucleotide sequence shown as SEQ ID NO:1.

Further provided by the invention is a substantially pure PAMP nucleic acid probe which contains substantially the nucleotide sequence of nucleotides 1 to 3221 of SEQ ID NO:1, or a fragment thereof, provided that the probe does not have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780.

The invention also provides a substantially pure PAMP nucleic acid probe which contains at least 10 contiguous nucleotides of SEQ ID NO:1, where the contiguous nucleotides include at least one nucleotide of the nucleotide sequence shown as position 1 to

position 3221 of SEQ ID NO:1, provided that the probe does not have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780. Such a PAMP nucleic acid probe can contain, for example, at least 15 contiguous nucleotides of SEQ ID NO:1, and can be, for example, 15 to 18 nucleotides in length. If desired, a substantially pure PAMP nucleic acid probe of the invention can further include a detectable label.

The invention also provides a substantially pure PAMP polypeptide which contains substantially the amino acid sequence shown as SEQ ID NO:2. In one embodiment, a substantially pure PAMP polypeptide of the invention has the amino acid sequence SEQ ID NO:2.

In addition, the invention provides a substantially pure PAMP polypeptide fragment, which includes at least eight contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2. Such a PAMP polypeptide fragment can include, for example, at least ten contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2.

The present invention provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual. The method is practiced by obtaining a sample from the individual; measuring a test expression level of PAMP RNA by hybridization with a PAMP nucleic acid probe comprising at least 10 contiguous nucleotides of SEQ ID NO:1, the contiguous nucleotides including at least one nucleotide

of the nucleotide sequence shown as position 1 to position 3221 of SEQ ID NO:1 in the sample; and comparing the test expression level of PAMP RNA to a control expression level of PAMP RNA, where a test expression level 2-fold or more greater than the control expression level indicates the presence of a prostate neoplastic condition. In a method of the invention, the sample can contain a prostate cell or a prostate tissue, and the control expression level can be determined using a normal prostate cell or an androgen-dependent cell line. The sample can be, for example, a fluid such as blood, serum, urine or semen. In one embodiment, the PAMP nucleic acid probe contains at least 10 contiguous nucleotides of SEQ ID NO:1, the contiguous nucleotides including at least one nucleotide of the nucleotide sequence shown as position 1 to position 3221 of SEQ ID NO:1, provided the probe does not have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780. A PAMP nucleic acid probe useful in a method of the invention can be, for example, 15 to 18 nucleotides in length and can contain, if desired, a detectable label.

The invention also provides a method of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual by obtaining a sample from the individual; measuring a test expression level of PAMP polypeptide by contacting a cell, a cell lysate, or fractionated sample thereof, from the individual with a binding agent selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2, and determining the amount of selective binding of the agent; and comparing the test expression level of PAMP

polypeptide to a control expression level of PAMP polypeptide, where a test expression level 2-fold or more greater than the control expression level indicates the presence of a prostate neoplastic condition. In a method of the invention, the binding agent selective for the PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2 can include, for example, an antibody, and can further include, if desired, a detectable label.

Further provided by the invention is a method of diagnosing metastatic prostate cancer in an individual by obtaining a sample from the individual, wherein the sample is not a prostate sample; measuring a test expression level of PAMP RNA by hybridization with a PAMP nucleic acid probe comprising at least 10 contiguous nucleotides of SEQ ID NO:1, the contiguous nucleotides including at least one nucleotide of the nucleotide sequence shown as position 1 to position 3221 of SEQ ID NO:1 in the sample; and comparing the test expression level of PAMP RNA to a control expression level of PAMP RNA, where a significant test expression level as compared to the control expression level indicates the presence of metastatic prostate cancer.

In addition, the invention provides a method of diagnosing metastatic prostate cancer in an individual by obtaining a sample from the individual, where the sample is not a prostate sample; measuring a test expression level of PAMP polypeptide by contacting a cell, a cell lysate, or fractionated sample thereof, from the individual with a binding agent selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2, and determining the amount of selective binding of the agent;

and comparing the test expression level of PAMP polypeptide to a control expression level of PAMP polypeptide, where a significant test expression level as compared to the control expression level indicates the presence of metastatic prostate cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the full-length nucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequence of PAMP. Predicted transmembrane domains are underlined.

Figure 2 shows northern analysis of PAMP expression in androgen-stimulated cells. Left panel: "+" indicates androgen-stimulated RNA; "-" indicates androgen-starved RNA. Right panel: A time course northern blot showing PAMP expression at 4, 8, 12, 16, 24, 36, 48 hours after androgen stimulation.

Figure 3 shows analysis of PAMP expression using two multiple tissue northern blots (MTN) from ClonTech (Palo Alto, CA).

Figure 4 shows hybridization of PAMP to a multiple tissue expression (MTE) array (ClonTech) containing 50 human tissues. The RNAs are as follows: A1, whole brain; A2, amygdala; A3, caudate nucleus; A4, cerebellum; A5, cerebral cortex; A6, frontal lobe; A7, hippocampus; A8, medulla oblongata; B1, occipital lobe; B2, putamen; B3; substantia nigra; B4, temporal lobe; B5, thalamus; B6, acumens; B7, spinal cord; C1, heart; C2, aorta; C3, skeletal muscle; C4, colon; C5, bladder; C6, uterus; C7, prostate; C8, stomach; D1, testis; D2, ovary;

D3, pancreas; D4, pituitary gland; D5, adrenal gland; D6, thyroid gland; D7, salivary gland; D8, mammary gland; E1, kidney; E2, liver; E3, small intestine; E4, spleen; E5, thymus; E6, peripheral leukocyte; E7, lymph node; E8, bone marrow; F1, appendix; F2, lung; F3, trachea; F4, placenta; G1, fetal brain; G2, fetal heart; G3, fetal kidney; G4, fetal liver; G5, fetal spleen; G6, fetal thymus; G7, fetal lung; H1, yeast total RNA; H2, yeast tRNA; H3, *E. Coli* rRNA; H4, *E. Coli* DNA; H5, poly r(A); H6, human C_0t 1 DNA; H7, human DNA; H8, human DNA; B8, F5-F8, G8 contain no RNAs.

Figure 5 shows RNA *in situ* hybridization with PAMP sense and antisense probes. A: Anti-sense probe of PAMP hybridized to a section of prostate cancer tissue sample. B: Sense probe of PAMP hybridized to prostate cancer section. C: Anti-sense probe of PAMP hybridized to normal prostate gland section. D: Sense probe of PAMP hybridized to normal prostate gland section.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to the discovery of the full-length coding sequence for PAMP, a gene with several transcripts expressed specifically in the prostate. The prostate-specific nucleic acid sequence and encoded gene product are useful as both diagnostic markers for neoplastic conditions of the prostate and as targets for therapy.

As disclosed herein in Example I, the PAMP cDNA contains 4485 nucleotides and is predicted to encode a protein of 1382 amino acids with at least 4 transmembrane

domains (see Figure 1). As further disclosed herein, PAMP expression is induced by androgen in the prostate carcinoma cell line LNCaP. Expression of the PAMP transcript was induced by 4 hours and maintained at least to 48 hours following androgen treatment (see Figure 2). As further disclosed herein, expression of the 2.0 and 3.2 kb PAMP transcripts was specific to prostate among 16 adult human tissues assayed by northern analysis (Figure 3), while the 5.0 and 6.5 kb forms were expressed in prostate, ovary and testis. Furthermore, among 50 human fetal and adult tissues assayed, significant expression was only detected in the prostate (see Figure 4). As further disclosed herein in Example III, RNA *in situ* analysis demonstrated that PAMP was expressed in epithelial cells in normal prostate and prostate cancer cells. These results demonstrate that PAMP is an androgen-regulated prostate-specific gene product.

Based on these results, the invention provides methods for diagnosing prostate neoplastic conditions. As discussed above, a PAMP gene of the invention is primarily expressed in prostate cells and becomes elevated in response to androgens. As such, a PAMP nucleic acid molecule or polypeptide of the invention can be used alone or in combination with other molecules as a specific marker for prostate cells and prostate neoplastic conditions.

PAMP Nucleic acid molecules

The present invention provides a PAMP nucleic acid molecule containing a nucleic acid sequence encoding substantially the amino acid sequence shown as SEQ ID

NO:2. A PAMP nucleic acid molecule can encode, for example, the amino acid sequence shown as SEQ ID NO:2 and, in one embodiment, contains the nucleotide sequence shown as SEQ ID NO:1.

The nucleic acid sequence of the PAMP cDNA (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO: 2) were determined as disclosed in Example I. As shown in Figure 1, the PAMP cDNA contains 4485 nucleotides and is predicted to encode a protein of 1382 amino acids.

The nucleic acid molecules of the invention and short oligonucleotide probes corresponding to unique sequences are useful in a variety of diagnostic procedures which employ probe hybridization methods. One advantage of employing nucleic acid hybridization in diagnostic procedures is that very small amounts of sample can be used because the analyte nucleic acid molecule can be amplified to many copies by, for example, polymerase chain reaction (PCR) or other well known methods for nucleic acid molecule amplification and synthesis.

As used herein, the term "nucleic acid molecule" is intended to mean a single- or double-stranded DNA or RNA molecule including, for example, genomic DNA, cDNA and mRNA. The term is intended to include nucleic acid molecules of both synthetic and natural origin. A nucleic acid molecule of natural origin can be derived from any animal, such as a human, non-human primate, mouse, rat, rabbit, bovine, porcine, ovine, canine, feline, or amphibian, or from a lower eukaryote. A nucleic acid molecule of the

invention can be of linear, circular or branched configuration, and can represent either the sense or antisense strand, or both, of a native nucleic acid molecule. A nucleic acid molecule of the invention can further incorporate a detectable moiety such as a radiolabel, a fluorochrome, a ferromagnetic substance, a luminescent tag or a detectable binding agent such as biotin.

As used herein, the term "substantially pure nucleic acid molecule" is intended to mean a nucleic acid molecule that is substantially free from cellular components or other contaminants that are not the desired molecule. A substantially pure nucleic acid molecule can also be sufficiently homogeneous so as to resolve as a band by gel electrophoresis, and generate a nucleotide sequence profile consistent with a predominant species.

<u>Nucleic acid probes</u>

A nucleic acid probe of the invention can contain substantially the nucleotide sequence of a portion of nucleotides 1 to 3221 of SEQ ID NO:1. The term "probe," as used herein in reference to a substantially pure nucleic acid molecule of the invention, is intended to refer to a portion of the nucleic acid molecule having the ability to selectively hybridize with the parent nucleic acid molecule. The term "selectively hybridize" refers to an ability to bind the parent nucleic acid molecule without substantial cross-reactivity with a molecule that is not the parent nucleic acid molecule. Therefore, the term includes specific hybridization where there is little or no

detectable cross-reactivity with other nucleic acid molecules. The term also includes minor cross-reactivity with other molecules provided hybridization to the subject nucleic acid molecule is distinguishable from hybridization to the cross-reactive species. Thus, a probe of the invention can be used, for example, as a PCR primer to selectively amplify a nucleic acid molecule of the invention; as a selective primer for 5' or 3' RACE to determine additional 5' or 3' sequence of a nucleic acid molecule of the invention; as a selective probe to identify or isolate a nucleic acid molecule of the invention on a RNA or DNA blot, or genomic or cDNA library; or as a selective inhibitor of transcription or translation of PAMP in a tissue, cell or cell extract. In one embodiment, the following sequences are excluded as nucleic acid probes of the invention: one or any combination of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780. In another embodiment, one or any combination of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201, nucleotides 1 to 1530 of AK026780, or nucleotides 1531 to 2000 of AK026780 is excluded from a probe of the invention. In a further embodiment, one or any combination of AA363808, AW959484, BE165930, BE893201, or AK026780, or a subsequence thereof containing at least ten contiguous nucleotides of any of these five sequences, is excluded from a probe of the invention.

Thus, the invention provides a substantially pure PAMP nucleic acid probe which contains substantially the nucleotide sequence of nucleotides 1 to 3221 of SEQ ID NO:1, or a fragment thereof, provided that the probe

does not have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780.

In one embodiment, the invention provides a substantially pure PAMP nucleic acid probe which contains at least 10 contiguous nucleotides of SEQ ID NO:1, where the contiguous nucleotides include at least one nucleotide of the nucleotide sequence shown as position 1 to position 3221 of SEQ ID NO:1, provided that the probe does not have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780. Such a PAMP nucleic acid probe can contain, for example, at least 15 contiguous nucleotides of SEQ ID NO:1, and can be, for example, 15 to 18 nucleotides in length. If desired, a substantially pure PAMP nucleic acid probe of the invention can further include a detectable label.

As used herein, the term "probe" refers to a portion of a subject nucleic acid molecule having at least 10 nucleotides.

A probe of the invention includes at least 10 contiguous nucleotides corresponding to the reference nucleic acid molecule, and can include at least 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or at least 25 nucleotides and, if desired, can include at least 30, 40, 50, 100, 300 or 500 nucleotides, and can include up to the full length of the reference nucleic acid molecule minus one nucleotide. Probes of such lengths are able to selectively hybridize with the subject nucleic acid

molecule in a variety of detection formats described herein.

As used herein, the term "substantially the nucleotide sequence" in reference to a nucleic acid molecule or nucleic acid probe of the invention includes sequences having one or more additions, deletions or substitutions with respect to the reference sequence, so long as the nucleic acid molecule retains its ability to selectively hybridize with the subject nucleic acid molecule.

Nucleic acid molecules and probes of the invention are useful as hybridization probes in diagnostic procedures. The probes can be as long as the full length transcript or as short as about 10-15 nucleotides, and preferably about 15-18 nucleotides. A probe of the invention can correspond to coding region or untranslated region sequence. The particular application and degree of desired specificity will be one consideration well known to those skilled in the art in selecting a probe. For example, if it is desired to detect PAMP and other related species, the probe can correspond to a coding sequence and be used in low stringency hybridization conditions. Alternatively, using high stringency conditions with a probe of the invention will select a PAMP nucleic acid molecule having substantially the nucleotide sequence shown as SEQ ID NO: 1. Untranslated region sequences corresponding to a PAMP transcript can also be used to construct probes since there is little evolutionary pressure to conserve non-coding domains. Probes as small as 15 nucleotides are statistically unique sequences within the human genome.

Therefore, fragments of the PAMP sequences of 15 nucleotides or more in length can be constructed from essentially any region of a PAMP cDNA, mRNA or promoter/regulatory region and be capable of uniquely hybridizing to PAMP DNA or RNA.

Nucleic acid probes can be produced recombinantly or chemically synthesized using methods well known in the art. Additionally, PAMP hybridization probes can be labeled with a variety of detectable labels including, for example, radioisotopes, fluorescent tags, reporter enzymes, biotin and other ligands. Such detectable labels can additionally be coupled with, for example, colorimetric or photometric indicator substrate for spectrophotometric detection. Methods for labeling and detecting such probes are well known in the art and can be found described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New York (1989), and Ausubel et al., Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999).

The nucleic acid probes of the invention can be hybridized under various stringency conditions readily determined by one skilled in the art. Depending on the particular assay, one skilled in the art can readily vary the stringency conditions to optimize detection of a PAMP nucleic acid molecule.

In general, the stability of a hybrid is a function of the ion concentration and temperature. Typically, a hybridization reaction is performed under conditions of lower stringency, followed by washes of

varying, but higher, stringency. Moderately stringent hybridization refers to conditions that permit a nucleic acid molecule such as a probe to bind a complementary nucleic acid molecule. The hybridized nucleic acid molecules generally have at least 60% identity, at least 75% identity, at least 85% identity; or at least 90% identity. Moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 42°C. High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at 65°C.

The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 22°C, followed by washing in 1X SSPE, 0.2% SDS, at 37°C. Denhart's solution contains 1% Ficoll, 1% polyvinylpyrolidone, and 1% bovine serum albumin (BSA). 20X SSPE (sodium chloride, sodium phosphate, ethylene diamide tetraacetic acid (EDTA)) contains 3M sodium chloride, 0.2M sodium phosphate, and 0.025 M (EDTA). Other suitable moderate stringency and high stringency hybridization buffers and conditions are well known to those of skill in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New York (1989); and Ausubel et al., *supra*, 1999). Nucleic acid molecules encoding polypeptides hybridize under moderately stringent or high stringency conditions to substantially the entire sequence, or substantial

portions, for example, typically at least 15-30 nucleotides of the nucleic acid sequence set forth in SEQ ID NO:1.

The invention also provides a modification of a PAMP nucleotide sequence that hybridizes to a PAMP nucleic acid molecule, for example, a nucleic acid molecule referenced as SEQ ID NO:1, under moderately stringent conditions. Modifications of PAMP nucleotide sequences, where the modification has at least 60% identity to a PAMP nucleotide sequence, are also provided. The invention also provides modification of a PAMP nucleotide sequence having at least 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, or at least 95% identity.

Identity of any two nucleic acid sequences can be determined by those skilled in the art based, for example, on a BLAST 2.0 computer alignment, using default parameters. BLAST 2.0 searching is available at http://www.ncbi.nlm.nih.gov/gorf/bl2.html., as described by Tatiana et al., <u>FEMS Microbiol Lett</u>. 174:247-250 (1999); Altschul et al., <u>Nucleic Acids Res.</u>, 25:3389-3402 (1997).

<u>PAMP polypeptides</u>

The invention also provides a substantially pure PAMP polypeptide which contains substantially the amino acid sequence shown as SEQ ID NO:2. In one embodiment, a substantially pure PAMP polypeptide of the invention has the amino acid sequence SEQ ID NO:2.

In addition, the invention provides a substantially pure PAMP polypeptide fragment, which includes at least eight contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2. Such a PAMP polypeptide fragment can include, for example, at least ten contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2.

In one embodiment, the invention provides a PAMP polypeptide fragment that includes at least eight contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2, provided that the fragment does not contain eight or more contiguous amino acids of AK026780. In a further embodiment, the invention provides a PAMP polypeptide fragment that includes at least eight contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2, provided that the fragment does not contain eight or more contiguous amino acids of AK026780, or eight or more contiguous amino acids encoded by any of the six reading frames of AA363808, AW959484, BE165930, BE893201 or AK026780.

Polypeptide fragments of the invention include peptides that can function as antigenic determinants to generate antibodies that are selective for a PAMP polypeptide encoded by substantially residues 1 to 1074 of SEQ ID NO:2.

Exemplary polypeptide fragments include those fragments having amino acids 1 to 8, 2 to 9, 3 to 10, etc. Other polypeptide fragments of residues 1 to 1074 shown in Figure 1 are also included as peptides that are potential antigenic fragments capable of eliciting an immune response to generate antibodies selective for PAMP

polypeptide residues 1 to 1074. It is understood that, while eight residues is the minimum length of a polypeptide fragment of the invention, a fragment can be longer and can include 9, 10, 11, 12, 13, 14, 15, 18, 20, 25, 30, 35, 40, 45 or more contiguous amino acids of residues 1 to 1074 of the PAMP polypeptide shown as SEQ ID NO:2.

The nucleic acid molecules and polypeptides of the invention encode a PAMP polypeptide. The term "PAMP polypeptide" as used herein, means a polypeptide that is structurally similar to human PAMP and that at least one biological activity of PAMP. Such a PAMP polypeptide has 50% or more sequence identity to SEQ ID NO:2, and can have 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to human PAMP (SEQ ID NO:2). Percent amino acid identity can be determined using Clustal W version 1.7 (Thompson et al., Nucleic Acids Res. 22:4673-4680 (1994))).

The present invention is directed to the full-length PAMP polypeptide sequence or large fragments of this full-length sequence. Thus, the term "PAMP polypeptide," as used herein, refers to a polypeptide corresponding to at least 350 of the 1382 residues of human PAMP. In view of the above, it is understood that a fragment containing, for example, residues 1075 to 1382 of SEQ ID NO:2 is not a "PAMP polypeptide" as defined herein.

Thus, it is clear to the skilled person that the term "PAMP polypeptide" encompasses polypeptides with one or more naturally occurring or non-naturally

occurring amino acid substitutions, deletions or insertions as compared to SEQ ID NO: 2, provided that the peptide has at least 50% amino acid identity with SEQ ID NO: 2 and corresponds to at least 350 residues of full-length PAMP. A PAMP polypeptide can be, for example, a naturally occurring variant of human PAMP (SEQ ID NO: 2), a species homolog including mammalian and non-mammalian homologs and murine, bovine, and primate homologs, a PAMP mutated by recombinant techniques, and the like. In view of the above, it is clear to the skilled person that the *Drosophila* polypeptide encoded by AAF57545.1 (CG11237) and the *C. elegans* polypeptide encoded by ZK520 (T27880), which each share 44% amino acid identity with human PAMP (SEQ ID NO:2) are not encompassed by the invention.

Modifications to SEQ ID NO: 2 that are encompassed within the invention include, for example, an addition, deletion, or substitution of one or more conservative or non-conservative amino acid residues; substitution of a compound that mimics amino acid structure or function; or addition of chemical moieties such as amino or acetyl groups.

In one embodiment, the invention provides a PAMP polypeptide having an amino acid sequence corresponding to at least 350 of the 1382 residues of human PAMP, provided that the polypeptide does not contain the sequence of AK026780. In another embodiment, the invention provides a PAMP polypeptides having an amino acid sequence corresponding to at least 350 of the 1382 residues of human PAMP, provided that the polypeptide does not contain the sequence of AK026780 or

the amino acid sequence encoded by any of the six reading frames of AA363808, AW959484, BE165930, BE893201 or AK026780.

The invention also provides antibodies that specifically bind a PAMP polypeptide. In one embodiment, the invention provides an antibody selective for PAMP polypeptide residues 1 to 1074, or a PAMP polypeptide fragment containing at least eight contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2.

As used herein, the term "antibody" is used in its broadest sense to include polyclonal and monoclonal antibodies, as well as antigen binding fragments of such antibodies. With regard to an antibody of the invention which is selective for PAMP polypeptide residues 1 to 1074 or a polypeptide fragment thereof, the term "antigen" means a native or synthesized fragment of PAMP residues 1 to 1074. Such an antibody of the invention, or antigen binding fragment of such an antibody, is characterized by having specific binding activity for PAMP polypeptide residues 1 to 1074, or a PAMP polypeptide fragment, of at least about 1×10^5 M^{-1}. Thus, Fab, $F(ab')_2$, Fd and Fv fragments of an anti-PAMP antibody, which retain specific binding activity for PAMP polypeptide residues 1 to 1074, or a polypeptide fragment thereof, are included within the definition of an antibody. Specific binding activity can be readily determined by one skilled in the art, for example, by comparing the binding activity of the antibody to PAMP polypeptide residues 1 to 1074 or a polypeptide fragment thereof versus a control polypeptide that does not include PAMP polypeptide residues 1 to 1074. Methods of

preparing polyclonal or monoclonal antibodies are well known to those skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988)).

In addition, the term "antibody" as used herein includes naturally occurring antibodies as well as non-naturally occurring antibodies, including, for example, single chain antibodies, chimeric, bifunctional and humanized antibodies, as well as antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, can be produced recombinantly or can be obtained, for example, by screening combinatorial libraries consisting of variable heavy chains and variable light chains as described by Huse et al. (Science 246:1275-1281 (1989)). These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bifunctional antibodies are well known to those skilled in the art (Winter and Harris, Immunol. Today 14:243-246 (1993); Ward et al., Nature 341:544-546 (1989) ; Harlow and Lane, *supra*, 1988); Hilyard et al., Protein Engineering: A practical approach (IRL Press 1992); Borrabeck, Antibody Engineering, 2d ed. (Oxford University Press 1995)).

An antibody of the invention can be prepared using as an immunogen a PAMP polypeptide, which can be prepared from natural sources or produced recombinantly, or a PAMP polypeptide fragment of the invention, which contains at least 8 contiguous amino acids of residues 1 to 1074 of SEQ ID NO:2. Such polypeptide fragments are functional antigenic fragments if the antigenic peptides

can be used to generate an antibody selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2. As is well known in the art, a non-immunogenic or weakly immunogenic PAMP polypeptide or polypeptide fragment can be made immunogenic by coupling the hapten to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various other carrier molecules and methods for coupling a hapten to a carrier molecule are well known in the art (see, for example, Harlow and Lane, *supra*, 1988). An immunogenic PAMP polypeptide fragment can also be generated by expressing the peptide portion as a fusion protein, for example, to glutathione S transferase (GST), polyHis or the like. Methods for expressing peptide fusions are well known to those skilled in the art (Ausubel et al., Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999)).

Diagnostic methods

Methods of diagnosing or predicting susceptibility to a prostate neoplastic condition in an individual further are provided by the invention. The methods of the invention are practiced by obtaining a sample from an individual; measuring a test expression level of PAMP in the sample; and comparing the test expression level of PAMP to a control expression level of PAMP, where a test expression level 2-fold or more greater than the control expression level indicates the presence of a prostate neoplastic condition. In a method of the invention, the sample can contain, for example, a prostate cell or prostate tissue and, in one embodiment, is a fluid such as blood, serum, urine or semen. The

control expression level can be determined, for example, using a normal prostate cell or an androgen-dependent cell line.

In a diagnostic method of the invention, a test expression level can be determined, for example, by measuring the amount of PAMP RNA and, in one embodiment, the amount of PAMP RNA is determined by hybridization with a PAMP nucleic acid probe containing at least 10 contiguous nucleotides of SEQ ID NO:1 and also including at least one nucleotide of the nucleotide sequence shown as position 1 to position 3221 of SEQ ID NO:1, provided that the probe does not have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780. A nucleic acid probe useful in a method of the invention can be, for example, 15 to 18 nucleotides in length, and, if desired, can further include a detectable label.

In a diagnostic method of the invention, a test expression level also can be determined, for example, by measuring the amount of PAMP polypeptide. In one embodiment, a diagnostic method of the invention is practiced by determining an amount of PAMP polypeptide by contacting a cell, a cell lysate, or fractionated sample thereof from the individual to be diagnosed with a binding agent selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2, and determining the amount of selective binding of the agent. A binding agent selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2 can be, for example, an antibody, and, if desired, can further include a detectable label.

As disclosed herein in Figure 4, PAMP was most highly expressed in prostate among 50 human tissues analyzed. These results indicate that PAMP expression outside of the prostate can be indicative of advanced prostate cancer, in which cancerous prostate cells have metastasized. Thus, the invention also provides a method of diagnosing metastatic prostate cancer in an individual by obtaining a sample from the individual, where the sample is not a prostate sample; measuring a test expression level of PAMP in the sample; and comparing the test expression level of PAMP to a control expression level of PAMP, where a significant test expression level as compared to the control expression level indicates the presence of metastatic prostate cancer.

As described herein, the term "prostate neoplastic condition" is intended to refer to a benign or malignant and metastatic prostate lesion of proliferating cells. For example, primary prostate tumors are classified into stages TX, T0, T1, T2, T3, and T4. Metastatic prostate cancer is classified into stages D1, D2, and D3. The term is also intended to include prostate neoplasm.

As used herein, the term "sample" is intended to mean any biological fluid, cell, tissue, organ or portion thereof, that includes or potentially includes nucleic acid molecules and polypeptides of the invention. The term includes samples present in an individual as well as samples obtained or derived from the individual. For example, a sample can be a histologic section of a specimen obtained by biopsy, or cells that are placed in or adapted to tissue culture. A sample further can be a

subcellular fraction or extract, or a crude or substantially pure nucleic acid molecule or protein preparation. A sample can be prepared by methods known in the art suitable for the particular format of the detection method.

As used herein, the term "detectable label" refers to a molecule that renders a nucleic acid molecule of the invention detectable by an analytical method. An appropriate detectable label depends on the particular assay format; such labels are well known by those skilled in the art. For example, a detectable label selective for a nucleic acid molecule can be a complementary nucleic acid molecule, such as a hybridization probe, that selectively hybridizes to the nucleic acid molecule. A hybridization probe can be labeled with a measurable moiety, such as a radioisotope, fluorochrome, chemiluminescent marker, biotin, or other moiety known in the art that is measurable by analytical methods. A detectable label also can be a nucleic acid molecule without a measurable moiety. For example, PCR or RT-PCR primers can be used without conjugation to selectively amplify all or a desired portion of the nucleic acid molecule. The amplified nucleic acid molecules can then be detected by methods known in the art.

As used herein, the term "binding agent" when used in reference to a PAMP polypeptide, is intended to mean a compound, including a simple or complex organic molecule, a metal containing compound, carbohydrate, peptide, protein, peptidomimetic, glycoprotein, lipoprotein, lipid, nucleic acid molecule, antibody, or the like that selectively binds a PAMP polypeptide or

PAMP polypeptide fragment, or to a PAMP gene regulatory sequence such as a promoter or enhancer element. For example, a binding agent can be a polypeptide that selectively binds with high affinity or avidity to a PAMP polypeptide, without substantial cross-reactivity with other polypeptides that are unrelated to a PAMP polypeptide. The affinity of a binding agent that selectively binds a PAMP polypeptide will generally be greater than about 10^5 M^{-1} and more preferably greater than about 10^6 M^{-1}. High affinity interactions can be preferred, and will generally be greater than about 10^8 M^{-1} to 10^9 M^{-1}. Specific examples of such selective binding agents include a polyclonal or monoclonal antibody selective for a PAMP polypeptide or peptide fragment thereof, nucleic acid molecule, nucleic acid analog, or small organic molecule, identified, for example, by affinity screening of a library. For certain applications, a binding agent can be utilized that preferentially recognizes a particular conformational or post-translationally modified state of a PAMP polypeptide. The binding agent can be labeled with a detectable moiety, if desired, or rendered detectable by specific binding to a detectable secondary binding agent.

As used herein, the term "test expression level" is used in reference to a PAMP mRNA or polypeptide expression and refers to the extent, amount or rate of synthesis of the nucleic acid sequence shown as SEQ ID NO: 1 or the PAMP polypeptide shown as SEQ ID NO: 2. The amount or rate of synthesis can be determined by measuring the accumulation or synthesis of PAMP RNA, PAMP polypeptide or by measuring an activity associated with a PAMP polypeptide.

In methods of the invention, the sample can be, for example, a prostate cell or prostate tissue such as a tissue biopsy. A sample can also be a fluid sample, for example, blood, serum, urine or semen. A normal sample can be, for example, a normal prostate cell or an androgen-dependent cell line.

In methods of the invention, a test expression level can be determined by measuring the amount of PAMP RNA, for example, by hybridization with a nucleic acid probe comprising substantially the nucleotide sequence of SEQ ID NO:1, or a fragment thereof. The probe can be, for example, an oligonucleotide of 15 to 18 nucleotides in length and, if desired, can contain a detectable label.

In one embodiment, a test expression level is determined by measuring the amount of PAMP RNA by hybridization with a PAMP nucleic acid probe that contains at least 10 contiguous nucleotides of SEQ ID NO:1, where the contiguous nucleotides include at least one nucleotide of the nucleotide sequence shown as position 1 to position 3221 of SEQ ID NO:1, provided that the probe does not have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780.

Alternatively, a test expression level can be determined by measuring the amount of PAMP polypeptide. The amount of PAMP polypeptide can be determined, for example, by contacting a cell, a cell lysate or fractionated sample thereof, from an individual with a binding agent selective for a PAMP polypeptide and

determining the amount of selective binding of the agent. The selective binding agent can be, for example, an antibody or other molecule identified as a PAMP polypeptide binding agent by the methods disclosed herein and, if desired, can contain a detectable label.

In one embodiment, a test expression is determined by measuring the amount of PAMP polypeptide using a binding agent selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2.

A prostate neoplastic condition is a benign or malignant prostate lesion of proliferating cells. Prostate neoplastic conditions include, for example, prostate interepithelial neoplasia (PIN) and prostate cancer. Prostate cancer is an uncontrolled proliferation of prostate cells which can invade and destroy adjacent tissues as well as metastasize. Primary prostate tumors can be classified into stages TX, T0, T1, T2, T3, and T4 and metastatic tumors can be classified into stages D1, D2 and D3. Similarly, there are classifications known by those skilled in the art for the progressive stages of precancerous lesions or PIN. The methods herein are applicable for the diagnosis or treatment of any or all stages of prostate neoplastic conditions.

The methods of the invention are also applicable to prostate pathologies other than neoplastic conditions. Such other pathologies include, for example, benign prostatic hyperplasia (BPH) and prostatitis. BPH is one of the most common diseases in adult males. Histological evidence of BPH has been found in more than 40% of men in their fifties and almost 90% of men in

their eighties. The disease results from the accumulation of non-malignant nodules arising in a small region around the proximal segment of the prostatic urethra which leads to an increase in prostate volume. If left untreated, BPH can result in acute and chronic retention of urine, renal failure secondary to obstructive uropathy, serious urinary tract infection and irreversible bladder decompensation. Prostatitis is an infection of the prostate. Other prostate pathologies known to those skilled in the art exist as well and are similarly applicable for diagnosis or treatment using the methods of the invention. Various neoplastic conditions of the prostate as well as prostate pathologies can be found described in, for example, Campbell's Urology, Seventh Edition, W.B. Saunders Company, Philadelphia (1998). Therefore, the methods of the invention are applicable to both prostate neoplastic conditions and prostate pathologies.

Therefore, the invention provides a method for both diagnosing and prognosing a prostate neoplastic condition including prostate cancer and prostate interepithelial neoplasia as well as other prostate pathologies such as BPH and prostatitis.

The invention also provides diagnostic methods relating to liver cancer. While PAMP expression is not observed in normal liver, expressed sequences corresponding to portions of PAMP have been isolated from tumor tissues such as hepatocellular carcinoma, and, to a lesser extent, invasive ovarian tumor, genitourinary tract tumors and endometrial adenocarcinoma. Furthermore, the 5.0 and 6.5 kb PAMP transcripts were

expressed and testis and ovary tissues (see Figure 3). These results indicate that elevated PAMP expression can be used to diagnose hepatocellular carcinoma, genitourinary tract tumors, endometrial adenocarcinoma, ovarian cancer and testicular cancer. One skilled in the art understands that the methods are practiced as described herein for prostate neoplastic conditions, with the sample chosen appropriately. For example, a sample containing liver cells is assayed according to a method of the invention for diagnosing or predicting susceptibility to liver cancer such as hepatocellular carcinoma.

Thus, the invention provides a method of diagnosing or predicting susceptibility to hepatocellular carcinoma in an individual by obtaining a sample from said individual; measuring a test expression level of PAMP in the sample; and comparing the test expression level of PAMP to a control expression level of PAMP, where a test expression level 2-fold or more greater than the control expression level indicates the presence of hepatocellular carcinoma.

The invention also provides a method of diagnosing or predicting susceptibility to ovarian cancer in an individual by obtaining a sample from said individual; measuring a test expression level of PAMP in the sample; and comparing the test expression level of PAMP to a control expression level of PAMP, where a test expression level 2-fold or more greater than the control expression level indicates the presence of ovarian cancer.

Thus, the invention provides a method of diagnosing or predicting susceptibility to testicular cancer in an individual by obtaining a sample from said individual; measuring a test expression level of PAMP in the sample; and comparing the test expression level of PAMP to a control expression level of PAMP, where a test expression level 2-fold or more greater than the control expression level indicates the presence of testicular cancer.

The invention provides a method of diagnosing or predicting prostate neoplastic conditions based on a finding of a positive correlation between a test expression level of PAMP in neoplastic cells of the prostate and the degree or extent of the neoplastic condition or pathology. The diagnostic methods of the invention are applicable to numerous prostate neoplastic conditions and pathologies as described above. One consequence of progression into these neoplastic and pathological conditions can be increased expression of PAMP in prostate tissue. The increase in PAMP expression in individuals suffering from a prostate neoplastic condition can be measured by comparing the amount of PAMP to that found, for example, in normal prostate tissue samples or in normal blood or serum samples. A two-fold or more increase in a test expression level in a prostate cell sample relative to a control expression sample obtained, for example, from normal prostate cells or from an androgen-dependent cell line is indicative of a prostate neoplastic condition or pathology. Similarly, an increase in PAMP expression leading to two-fold or more secretion into the blood or other circulatory fluids of the individual compared to control blood or fluid

samples also can be indicative of a prostate neoplastic condition or pathology.

As a diagnostic indicator, PAMP can be used qualitatively to positively identify a prostate neoplastic condition or pathology as described above. Alternatively, PAMP also can be used quantitatively to determine the degree or susceptibility of a prostate neoplastic condition or pathology. For example, successive increases in the expression levels of PAMP can be used as a predictive indicator of the degree or severity of a prostate neoplastic condition or pathology. For example, increased expression can lead to a rise in accumulated levels and can be positively correlated with increased severity of a neoplastic condition of the prostate. A higher level of PAMP expression can be correlated with a later stage of a prostate neoplastic condition or pathology. For example, increases in expression levels of two-fold or more compared to a normal sample can be indicative of at least prostate neoplasia. PAMP also can be used quantitatively to distinguish between pathologies and neoplastic conditions as well as to distinguish between the different types of neoplastic conditions.

Correlative increases can be determined by comparison of PAMP expression from the individual having, or suspected of having, a neoplastic condition of the prostate to expression levels of PAMP from known samples determined to exhibit a prostate neoplastic condition. Alternatively, correlative increases also can be determined by comparison of a test expression level of PAMP expression to expression levels of other known

markers of prostate cancer such as prostate specific antigen (PSA), glandular kallikrein 2 (hK2) and prostase/PRSS18. These other known markers can be used, for example, as an internal or external standard for correlation of stage-specific expression with increases in PAMP expression and severity of the neoplastic or pathological condition. Conversely, a regression in the severity of a prostate neoplastic condition or pathology can be followed by a corresponding decrease in PAMP expression levels and can similarly be assessed using the methods described herein.

Given the teachings and guidance provided herein, those skilled in the art will know or can determine the stage or severity of a prostate neoplastic condition or pathology based on a determination of PAMP expression and correlation with a prostate neoplastic condition or pathology. A correlation can be determined using known procedures and marker comparisons as described herein. For a review of recognized values for such other marker in normal versus pathological tissues, see, for example, Campbell's Urology, Seventh Edition, W.B. Saunders Company, Philadelphia (1998).

The use of PAMP expression levels in prostate cells, the circulatory system and urine as a diagnostic indicator of a prostate pathology allows for early diagnosis as a predictive indicator when no physiological or pathological symptoms are apparent. The methods are particularly applicable to any males over age 50, African-American males and males with familial history of prostate neoplastic conditions or pathologies. The diagnostic methods of the invention also are particularly

applicable to individuals predicted to be at risk for prostate neoplastic conditions or pathologies by reliable prognostic indicators prior to onset of overt clinical symptoms. All that is necessary is to determine the PAMP prostate tissue or circulatory or bodily fluid expression levels to determine whether there is an increase in these PAMP levels in the individual suspected of having a prostate pathology compared to a control expression level such as the level observed in normal individuals. Those skilled in the art will know by using routine examinations and practices in the field of medicine those individuals who are applicable candidates for diagnosis by the methods of the invention.

For example, individuals suspected of having a prostate neoplastic condition or pathology can be identified by exhibiting presenting signs of prostate cancer which include, for example, a palpable nodule (> 50% of the cases), dysuria, cystitis and prostatitis, frequency, urinary retention, or decreased urine stream. Signs of advanced disease include pain, uremia, weight loss and systemic bleeding. Prognostic methods of this invention are applicable to individuals after diagnosis of a prostate neoplastic condition, for example, to monitor improvements or identify residual neoplastic prostate cells using, for example, imaging methods known in the art and which target PAMP.

Therefore, the invention also provides a method of predicting the onset of a prostate neoplastic condition or pathology. The method consists of determining increased PAMP expression levels in a prostate cell sample or in fluids from an individual

having or suspected of having a prostate neoplastic condition or pathology compared to a sample isolated from a normal individual, where increased PAMP expression in the sample indicates the onset of the prostate neoplastic condition or pathology.

The diagnostic methods of the invention are applicable for use with a variety of different types of samples isolated or obtained from an individual having, or suspected of having a prostate neoplastic condition or prostate pathology. For example, samples applicable for use in one or more diagnostic formats of the invention, include tissue and cell samples. A tissue or cell sample can be obtained, for example, by biopsy or surgery. As described below, and depending on the format of the method, the tissue can be used whole or subjected to various methods known in the art to disassociate the sample into smaller pieces, cell aggregates or individual cells. Additionally, when combined with amplification methods such as polymerase chain reaction (PCR), a single prostate cell sample is sufficient for use in diagnostic assays of the invention which employ hybridization detection methods. Similarly, when measuring PAMP polypeptide or activity levels, amplification of the signal with enzymatic coupling or photometric enhancement can be employed using only a few or a small number of cells.

Whole tissue obtained from a prostate biopsy or surgery is one example of a prostate cell sample. Whole tissue prostate cell samples can be assayed employing any of the formats described below. For example, the prostate tissue sample can be mounted and hybridized *in*

situ with PAMP nucleic acid probes. Similar histological formats employing protein detection methods and *in situ* activity assays also can be used to detect a PAMP polypeptide in whole tissue prostate cell samples. Protein detection methods include, for example, staining with a PAMP specific antibody and activity assays. Such histological methods as well as others well known to those skilled in the art are applicable for use in the diagnostic methods of the invention using whole tissue as the source of a prostate cell sample. Methods for preparing and mounting the samples are similarly well known in the art.

Individual prostate cells and cell aggregates from an individual having, or suspected of having a prostate neoplastic condition or pathology is another example of a prostate cell sample which can be analyzed for increased expression of PAMP RNA, polypeptide or activity. The cells can be grown in culture and analyzed *in situ* using procedures such as those described above. Whole cell samples expressing cell surface markers associated with PAMP expression can be rapidly tested using fluorescent or magnetic activated cell sorting (FACS or MACS) with labeled binding agents selective for the surface marker or using binding agents selective for epithelial or prostate cell populations, for example, and then determining a test expression level of PAMP within this population. The test expression level can be determined using, for example, binding agents selective for PAMP or by hybridization to a PAMP specific probe. Other methods for measuring the expression level of PAMP in whole cell samples are known in the art and are

similarly applicable in any of the diagnostic formats described below.

The tissue or whole cell prostate cell sample obtained from an individual also can be analyzed for increased PAMP expression by lysing the cell and measuring a test expression levels of PAMP in the lysate, a fractionated portion thereof or a purified component thereof using any of diagnostic formats described herein. For example, if a hybridization format is used, PAMP RNA can be amplified directly from the lysate using PCR, or other amplification procedures well known in the art such as RT-PCR, 5' or 3' RACE to directly measure the expression levels of PAMP nucleic acid molecules. RNA also can be isolated and probed directly such as by solution hybridization or indirectly by hybridization to immobilized RNA. Similarly, when determining a test expression level of PAMP using polypeptide detection formats, lysates can be assayed directly, or they can be further fractionated to enrich for PAMP and its corresponding activity. Numerous other methods applicable for use with whole prostate cell samples are well known to those skilled in the art and can accordingly be used in the methods of the invention.

The prostate tissue or cell sample can be obtained directly from the individual or, alternatively, it can be obtained from other sources for testing. Similarly, a cell sample can be tested when it is freshly isolated or it can be tested following short or prolonged periods of cryopreservation without substantial loss in accuracy or sensitivity. If the sample is to be tested following an indeterminate period of time, it can be

obtained and then cryopreserved, or stored at 4°C for short periods of time, for example. An advantage of the diagnostic methods of the invention is that they do not require histological analysis of the sample. As such, the sample can be initially disaggregated, lysed, fractionated or purified and the active component stored for later diagnosis.

The diagnostic methods of the invention are applicable for use with a variety of different types of samples other than prostate cell samples. For example, a PAMP polypeptide or fragment thereof that is released into the extracellular space, including circulatory fluids as well as other bodily fluids, can be used in diagnostic methods to detect a secreted polypeptide or fragment related to a PAMP polypeptide. In such a case, the diagnostic methods of the invention are applicable with fluid samples collected from an individual having, or suspected of having a neoplastic condition of the prostate or a prostate pathology.

Fluid samples, which can be measured for PAMP expression levels, include, for example, blood, serum, lymph, urine and semen. Other bodily fluids are known to those skilled in the art and are similarly applicable for use as a sample in the diagnostic methods of the invention. One advantage of analyzing fluid samples is that they are readily obtainable, in sufficient quantity, without invasive procedures as required by biopsy and surgery. Analysis of fluid samples such as blood, serum and urine will generally be in the diagnostic formats described herein which measure PAMP polypeptide levels or activity. As the PAMP related polypeptide is circulating

in a soluble form, the methods will be similar to those which measure expression levels from cell lysates, fractionated portions thereof or purified components.

Prostate neoplastic conditions and prostate pathologies can be diagnosed, predicted or prognosed by measuring a test expression level of PAMP in a prostate cell sample, circulating fluid or other bodily fluid obtained from the individual. As described herein, expression levels can be measured by a variety methods known in the art. For example, a test expression level of PAMP can be determined by measuring the amount of PAMP RNA or polypeptide in a sample from the individual. Alternatively, a test expression level of PAMP can be determined by measuring the amount of a PAMP activity in the sample, the amount of activity being indicative of PAMP expression levels.

One skilled in the art can readily determine an appropriate assay system given the teachings and guidance provided herein and choose a method based on measuring RNA, polypeptide or activity. Considerations such as the sample type, availability and amount will also influence selection of a particular diagnostic format. For example, if the sample is a prostate cell sample and there is only a small amount available, then diagnostic formats which measure the amount of PAMP RNA by, for example, PCR amplification, or which measure PAMP-related cell surface polypeptide by, for example, FACS analysis can be appropriate choices for determining the expression level of PAMP. Alternatively, if the sample is a blood sample and the user is analysing numerous different samples simultaneous, such as in a clinical setting, then

a multisample format, such as an Enzyme Linked Immunoabsorbant Assay (ELISA), which measures the amount of PAMP polypeptide can be an appropriate choice for determining the expression level of PAMP. Additionally, PAMP nucleic acid molecules released into bodily fluids from the neoplastic or pathological prostate cells can also be analyzed by, for example, PCR or RT-PCR. Those skilled in the art will know, or can determine which format is amenable for a particular application and which methods or modifications known within the art are compatible with a particular type of format.

Hybridization methods are applicable for measuring the amount of PAMP RNA as an indicator of PAMP expression levels. There are numerous methods well known in the art for detecting nucleic acid molecules by specific or selective hybridization with a complementary probe. Such methods include both solution hybridization procedures and solid-phase hybridization procedures where the probe or sample is immobilized to a solid support. Descriptions for such methods can be found in, for example, Sambrook et al., *supra*, and in Ausubel et al., *supra*. Specific examples of such methods include PCR and other amplification methods such as RT-PCR, 5' or 3' RACE, RNase protection, RNA blot, dot blot or other membrane-based technologies, dip stick, pin, ELISA or two-dimensional arrays immobilized onto chips as a solid support. These methods can be performed using either qualitative or quantitative measurements, all of which are well known to those skilled in the art.

PCR or RT-PCR can be used with isolated RNA or crude cell lysate preparations. As described previously, PCR is advantageous when there is limiting amounts of starting material. A further description of PCR methods can be found in, for example, Dieffenbach, C.W., and Dveksler, G.S., PCR Primer: A Laboratory Manual, Cold Spring Harbor Press, Plainview, New York (1995). Multisample formats such as an ELISA or two-dimensional array offer the advantage of analyzing numerous, different samples in a single assay. A particular example of a two-dimentional array used in a hybridization format is described further below in the Examples. In contrast, solid-phase dip stick-based methods offer the advantage of being able to rapidly analyze a patient's fluid sample and obtain an immediate result.

Nucleic acid probes useful for measuring the expression level of PAMP by hybridization include, for example, all of the PAMP nucleic acid probes described herein. More specifically, such probes include, for example, nucleic acid molecules corresponding to the entire PAMP cDNA (SEQ ID NO:1) and fragments thereof. Smaller fragments thereof also can be used, including oligonucleotides corresponding to PAMP nucleotide sequences and which are capable of specifically or selectively hybridizing to PAMP RNA. In a preferred embodiment, the diagnostic methods of the invention employ a PAMP nucleic acid probe that contains at least 10 contiguous nucleotides of SEQ ID NO:1, where the contiguous nucleotides include at least one nucleotide of the nucleotide sequence shown as position 1 to position 3221 of SEQ ID NO:1, provided that the probe does not

have the nucleotide sequence of AA363808, AW959484, BE165930, nucleotides 1 to 614 of BE893201 or nucleotides 1 to 1530 of AK026780.

Briefly, for detection by hybridization, the PAMP nucleic acid probes having detectable labels are added to a prostate cell sample or a fluid sample obtained from the individual having, or suspected of having a prostate neoplastic condition or pathology under conditions which allow annealing of the probe to PAMP RNA. Methods for detecting PAMP RNA in a sample can include the use of, for example, RT-PCR. Conditions are well known in the art for both solution and solid phase hybridization procedures. Moreover, optimization of hybridization conditions can be performed, if desired, by hybridization of an aliquot of the sample at different temperatures, durations and in different buffer conditions. Such procedures are routine and well known to those skilled. Following annealing, the sample is washed and the signal is measured and compared with a suitable control or standard value. The magnitude of the hybridization signal is directly proportional to the expression levels of PAMP.

A test expression level is compared to a suitable control expression level, which can be, for example, the expression level of PAMP from a prostate cell or a fluid sample obtained from a normal individual. Another suitable control for comparison is a prostate cell line that is androgen-dependent. PAMP expression levels in cell lines should be determined under androgen depleted growth conditions, as their response to androgen stimulation will be indicative of PAMP expression levels

in neoplastic cells. The control expression level can be determined simultaneously with one or more test samples or, alternatively, expression levels can be established for a particular type of sample and standardized to internal or external parameters such as protein or nucleic acid content, cell number or mass of tissue. Such standardized control samples can then be directly compared with results obtained from the test sample. An increase of two-fold or more of a test expression level of PAMP indicates the presence of a prostate neoplastic condition or pathology in the tested individual.

The diagnostic procedures described herein can additionally be used in conjunction with other prostate markers, such as prostate specific antigen, human glandular kallikrein 2 (hk2) and prostase/PRSS18 for simultaneous or independent corroboration of a sample. Additionally, PAMP can be used, for example, in combination with other markers to further distinguish normal basal cells, secretory cells and neoplastic cells of the prostate. Moreover, PAMP expression can be used in conjunction with smooth muscle cell markers to distinguish between pathological conditions such as benign prostate hypertrophy (BPH) and neoplasia. Those skilled in the art will know which markers are applicable for use in conjunction with PAMP to delineate more specific diagnostic information such as that described above.

The invention additionally provides a method of diagnosing or predicting the susceptibility of a prostate neoplastic condition in an individual suspected of having a neoplastic condition of the prostate where PAMP

expression level is determined by measuring the amount of PAMP polypeptide. The method consists of contacting a cell, a cell lysate, or fractionated sample thereof, from the individual with a binding agent selective for PAMP, and determining the amount of selective binding of the agent. In one embodiment, the binding agent is an antibody.

In one embodiment, a test expression level is determined by measuring the amount of PAMP polypeptide using a binding agent selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2. In a further embodiment, a test expression level is determined by measuring the amount of PAMP polypeptide using an antibody selective for PAMP polypeptide residues 1 to 1074 of SEQ ID NO:2.

Essentially all modes of affinity binding assays are applicable for use in determining a test expression level of a PAMP polypeptide in a sample. Such methods are rapid, efficient and sensitive. Moreover, affinity binding methods are simple and can be modified to be performed under a variety of clinical settings and conditions to suit a variety of particular needs. Affinity binding assays which are known and can be used in the methods of the invention include both soluble and solid phase formats. A specific example of a soluble phase affinity binding assay is immunoprecipitation using a PAMP selective antibody or other binding agent. Solid phase formats are advantageous for the methods of the invention since they are rapid and can be performed more easily on multiple different samples simultaneously without losing sensitivity or accuracy. Moreover, solid

phase affinity binding assays are further amenable to high throughput screening and automation.

Specific examples of solid phase affinity binding assays include immunoaffinity binding assays such as an ELISA and radioimmune assay (RIA). Other solid phase affinity binding assays are known to those skilled in the art and are applicable to the methods of the invention. Although affinity binding assays are generally formatted for use with an antibody binding molecules that is selective for the analyte or ligand of interest, essentially any binding agent can be alternatively substituted for the selectively binding antibody. Such binding agents include, for example, macromolecules such as polypeptides, peptides, nucleic acid molecules, lipids and sugars as well as small molecule compounds. Methods are known in the art for identifying such molecules which bind selectively to a particular analyte or ligand and include, for example, surface display libraries and combinatorial libraries. Thus, for a molecule other than an antibody to be used in an affinity binding assay, all that is necessary is for the binding agent to exhibit selective binding activity for PAMP.

Various modes of affinity binding formats are similarly known which can be used in the diagnostic methods of the invention. For the purpose of illustration, particular embodiments of such affinity binding assays will be described further in reference to immunoaffinity binding assays. The various modes of affinity binding assays, such as immunoaffinity binding assays, include, for example, solid phase ELISA and RIA

as well as modifications thereof. Such modifications thereof include, for example, capture assays and sandwich assays as well as the use of either mode in combination with a competition assay format. The choice of which mode or format of immunoaffinity binding assay to use will depend on the intent of the user. Such methods can be found described in common laboratory manuals such as Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York (1999).

As with the hybridization methods described previously, the diagnostic formats employing affinity binding can be used in conjunction with a variety of detection labels and systems known in the art to quantitate amounts of PAMP in the analyzed sample. Detection systems include the detection of bound PAMP by both direct and indirect means. Direct detection methods include labeling of the PAMP-selective antibody or binding agent. Indirect detection systems include, for example, the use of labeled secondary antibodies and binding agents.

Secondary antibodies, labels and detection systems are well known in the art and can be obtained commercially or by techniques well known in the art. The detectable labels and systems employed with the PAMP-selective binding agent should not impair binding of the agent to PAMP. Moreover, multiple antibody and label systems can be employed for detecting the bound PAMP-selective antibody to enhance the sensitivity of the binding assay if desired.

As with the hybridization formats described previously, detectable labels can be essentially any label that can be quantitated or measured by analytical methods. Such labels include, for example, enzymes, radioisotopes, fluorochromes as well as chemi- and bioluminescent compounds. Specific examples of enzyme labels include horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, urease and luciferase.

A horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble product in the presence of hydrogen peroxide that is detectable by measuring absorbance at 450 nm. An alkaline phosphatase detection system can be used with the chromogenic substrate *p*-nitrophenyl phosphate, for example, which yields a soluble product readily detectable by measuring absorbance at 405 nm. Similarly, a β-galactosidase detection system can be used with the chromogenic substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG), which yields a soluble product detectable by measuring absorbance at 410 nm, or a urease detection system can be used with a substrate such as urea-bromocresol purple (Sigma Immunochemicals, St. Louis, MO). Luciferin is the substrate compound for luciferase which emits light following ATP-dependent oxidation.

Fluorochrome detection labels are rendered detectable through the emission of light of ultraviolet or visible wavelength after excitation by light or another energy source. DAPI, fluorescein, Hoechst 33258, R-phycocyanin, B-phycoerythrin, R-phycoerythrin,

rhodamine, Texas red and lissamine are specific examples of fluorochrome detection labels that can be utilized in the affinity binding formats of the invention. A particularly useful fluorochrome is fluorescein or rhodamine.

Chemiluminescent as well as bioluminescent detection labels are convenient for sensitive, non-radioactive detection of PAMP and can be obtained commercially from various sources such as Amersham Lifesciences, Inc. (Arlington Heights, IL).

Alternatively, radioisotopes can be used as detectable labels in the methods of the invention. Iodine-125 is a specific example of a radioisotope useful as a detectable label.

Signals from detectable labels can be analyzed, for example, using a spectrophotometer to detect color from a chromogenic substrate; a fluorometer to detect fluorescence in the presence of light of a certain wavelength; or a radiation counter to detect radiation, such as a gamma counter for detection of iodine-125. For detection of an enzyme-linked secondary antibody, for example, a quantitative analysis of the amount of bound agent can be made using a spectrophotometer such as an EMAX Microplate Reader (Molecular Devices, Menlo Park, CA) in accordance with the manufacturer's instructions. If desired, the assays of the invention can be automated or performed robotically, and the signal from multiple samples can be detected simultaneously.

The diagnostic formats of the present invention can be forward, reverse or simultaneous as described in U.S. Patent No. 4,376,110 and No. 4,778,751. Separation steps for the various assay formats described herein, including the removal of unbound secondary antibody, can be performed by methods known in the art (Harlow and Lane, *supra*). For example, washing with a suitable buffer can be followed by filtration, aspiration, vacuum or magnetic separation as well as by centrifugation.

A binding agent selective for PAMP also can be utilized in imaging methods that are targeted at PAMP expressing prostate cells. These imaging techniques will have utility in identification of residual neoplastic cells at the primary site following standard treatments including, for example, radical prostatectomy, radiation or hormone therapy. In addition, imaging techniques that detect neoplastic prostate cells have utility in detecting secondary sites of metastasis. The PAMP specific binding agent can be radiolabeled with, for example, ^{111}indium and infused intravenously as described by Kahn et al., Journal of Urology 152:1952-1955 (1994). The binding agent selective for PAMP can be, for example, a monoclonal antibody selective for PAMP polypeptide. Imaging can be accomplished by, for example, radioimmunoscintigraphy as described by Kahn et al., *supra*.

The invention additionally provides a method of diagnosing or predicting the susceptibility of a prostate neoplastic condition in an individual suspected of having a neoplastic condition of the prostate, where a test expression level of PAMP is determined by measuring the

amount of PAMP activity. The method consists of contacting a cell, a cell lysate, or fractionated sample thereof, from the individual with an agent that functions to measure an activity associated with PAMP.

Another diagnostic format which can be used for determining the expression levels of PAMP is by measuring an activity associated with a PAMP polypeptide. As with the hybridization and affinity binding formats, activity assays can similarly be performed using essentially identical methods and modes of analysis. Therefore, solution and solid phase modes, including multisample ELISA, RIA and two-dimentional array procedures are applicable for use in measuring an activity associated with PAMP. The activity can be measured by, for example, incubating an agent that functions to measure an activity associated with PAMP with the sample and determining the amount of product formed that corresponds to a PAMP activity. The amount of product formed will directly correlate with the PAMP activity in the sample and therefore, with the expression levels of PAMP in the sample.

The invention further provides a method of identifying a compound that inhibits the PAMP activity. The method consists of contacting a sample containing PAMP and an agent that functions to measure an activity associated with PAMP with a test compound under conditions that allow formation of a product that corresponds to a PAMP activity and measuring the amount of product formed, wherein a decrease in the amount of product formed in the presence of the test compound compared to the absence of the test compound indicates

that the compound has PAMP inhibitory activity. Similarly, compounds that increase the activity of PAMP also can be identified. A test compound added to a sample containing PAMP and an agent that functions to measure an activity associated with PAMP which increases the amount of product formed compared to the absence of the test compound indicates that the compound increases PAMP activity. Therefore, the invention provides a method of identifying compounds that modulate the activity of PAMP. The PAMP containing sample used for such a method can be serum, prostate tissue, a prostate cell population or a recombinant cell population expressing PAMP.

Those compounds having inhibitory activity are considered as potential PAMP antagonists and further as potential therapeutic agents for treatment of neoplastic conditions of the prostate. Similarly, those compounds which increase a PAMP activity are considered as potential PAMP agonists and further as potential therapeutic agents for the treatment of neoplastic conditions of the prostate.

Within the biological arts, the term "about" when used in reference to a particular activity or measurement is intended to refer to the referenced activity or measurement as being within a range of values encompassing the referenced value and within accepted standards of a credible assay within the art, or within accepted statistical variance of a credible assay within the art.

A reaction system for identifying a compound that inhibits or enhances PAMP activity can be performed using essentially any source of PAMP activity. Such sources include, for example, a prostate cell sample, lysate or fractionated portion thereof; a bodily fluid such as blood, serum or urine from an individual with a prostate neoplastic condition; a recombinant cell or soluble recombinant source, and an *in vitro* translated source. The PAMP source is combined with an agent that functions to measure an activity associated with PAMP as described above and incubated in the presence or absence of a test inhibitory compound. The amount of product that corresponds to a PAMP activity that is formed in the presence of the test compound is compared with that in the absence of the test compound. Those test compounds which provide inhibition of product formation of at least about 50% are considered to be PAMP inhibitors. Similarly, those compounds which increase product formation of two-fold or more are considered to be PAMP enhancers or activators. PAMP inhibitors can then be subjected to further *in vitro* or *in vivo* testing to confirm that they inhibit a PAMP activity in cellular and animal models.

Suitable test compounds for the inhibition or enhancement assays can be any substance, molecule, compound, mixture of molecules or compounds, or any other composition which is suspected of being capable of inhibiting PAMP activity *in vivo* or *in vitro*. The test compounds can be macromolecules, such as biological polymers, including proteins, polysacchrides and nucleic acid molecules. Sources of test compounds which can be screened for PAMP inhibitory activity include, for

example, libraries of peptides, polypeptides, DNA, RNA and small organic compounds. The test compounds can be selected randomly and tested by the screening methods of the present invention. Test compounds are administered to the reaction system at a concentration in the range from about 1 nM to 1 mM.

Methods for producing pluralities of compounds to use in screening for compounds that modulate the activity of a PAMP polypeptide, including chemical or biological molecules that are inhibitors or enhancers of PAMP activity such as simple or complex organic molecules, metal-containing compounds, carbohydrates, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, nucleic acid molecules, antibodies, and the like, are well known in the art and are described, for example, in Huse, U.S. Patent No. 5,264,563; Francis et al., Curr. Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr. Biol., 2:363-371 (1998); Sofia, Mol. Divers. 3:75-94 (1998); Eichler et al., Med. Res. Rev. 15:481-496 (1995); and the like. Libraries containing large numbers of natural and synthetic compounds also can be obtained from commercial sources. Combinatorial libraries of molecules can be prepared using well known combinatorial chemistry methods (Gordon et al., J. Med. Chem. 37: 1233-1251 (1994); Gordon et al., J. Med. Chem. 37: 1385-1401 (1994); Gordon et al., Acc. Chem. Res. 29:144-154 (1996); Wilson and Czarnik, eds., Combinatorial Chemistry: Synthesis and Application, John Wiley & Sons, New York (1997)).

Therefore, the invention provides a method of identifying a compound that inhibits or enhances a PAMP activity where the sample further consists of a prostate cell lysate, a recombinant cell lysate expressing PAMP, an *in vitro* translation lysate containing PAMP mRNA, a fractionated sample of a prostate cell lysate, a fractionated sample of a recombinant cell lysate expressing PAMP, a fractionated sample of an *in vitro* translation lysate containing PAMP mRNA or an isolated PAMP polypeptide. The method can be performed in single or multiple sample format.

In another embodiment, PAMP polypeptides and PAMP peptides can be used as vaccines to prophylactically treat individuals for the occurrence of a prostate neoplastic condition or pathology. Such vaccines can be used to induce B or T cell immune responses or both aspects of the individuals endogenous immune mechanisms. The mode of administration and formulations to induce either or both of these immune responses are well known to those skilled in the art. For example, PAMP polypeptides and peptides can be administered in many possible formulations, including pharmaceutically acceptable mediums. They can be administered alone or, for example, in the case of a peptide, the peptide can be conjugated to a carrier, such as KLH, in order to increase its immunogenicity. The vaccine can include or be administered in conjunction with an adjuvant, various of which are known to those skilled in the art. After initial immunization with the vaccine, further boosters can be provided if desired. Therefore, the vaccines are administered by conventional methods in dosages which are sufficient to elicit an immunological response, which can

be easily determined by those skilled in the art. Alternatively, the vaccines can comprise anti-idiotypic antibodies which are internal images of the PAMP polypeptides and peptides described above. Methods of making, selecting and administering such anti-idiotype vaccines are well known in the art. See, for example, Eichmann, et al., CRC Critical Reviews in Immunology 7:193-227 (1987). In addition, the vaccines can comprise a nucleic acid molecule encoding PAMP, for example, substantially the nucleotide sequence shown as SEQ ID NO:1. Methods for using nucleic acid molecules such as DNA as vaccines are well known to those skilled in the art (see, for example, Donnelly et al. (<u>Ann. Rev. Immunol.</u> 15:617-648 (1997)); Felgner et al. (U.S. Patent No. 5,580,859, issued December 3, 1996); Felgner (U.S. Patent No.5,703,055, issued December 30, 1997); and Carson et al. (U.S. Patent No. 5,679,647, issued October 21, 1997)).

The invention additionally provides a method of treating or reducing the progression of a prostate neoplastic condition. The method consists of administering to an individual having a neoplastic condition of the prostate an inhibitory amount of a PAMP specific inhibitor, wherein said inhibitory amount causes a reduction of at least about 2-fold in the amount or activity of PAMP. A specific example of a PAMP specific inhibitor is a PAMP nucleic acid molecule. PAMP inhibitors, including antibodies, antisense nucleic acid molecules and compounds identified by the methods described herein can therefore be used as therapeutics for treating or reducing the severity of an individual with a prostate neoplastic condition or pathology.

As used herein, the term "inhibitor" is intended to refer to an agent effecting a decrease in the extent, amount or rate of PAMP expression or effecting a decrease in the activity of a PAMP polypeptide activity. An example of a PAMP inhibitor which effects a decrease in PAMP expression includes PAMP antisense nucleic acid molecules and transcriptional inhibitors that bind to the PAMP 5' promoter/regulatory region.

As used herein, the term "inhibitory amount" is intended to refer to the amount of an inhibitor necessary to effect a reduction of at least about 2-fold in the extent, amount or rate of PAMP expression.

Such inhibitors can be produced using methods which are generally known in the art, and include the use of purified PAMP polypeptide to produce antibodies or to screen libraries of compounds, as described previously, for those which specifically bind PAMP. For example, in one aspect, antibodies which are selective for PAMP can be used directly as an antagonist, or indirectly as a targeting or delivery mechanism for bringing a cytotoxic or cytostatic agent to neoplastic prostate cells. Such agents can be, for example, radioisotopes. The antibodies can be generated using methods that are well known in the art and include, for example, polyclonal, monoclonal, chimeric, humanized single chain, Fab fragments, and fragments produced by a Fab expression library.

As used herein, the term "functional fragment" when used in reference to a 5' promoter and regulatory region of PAMP is intended to refer to a portion of the

promoter and regulatory region having at least one of the activities of its parent nucleic acid molecule. For example, a functional fragment can be a transcriptional regulatory element in a PAMP gene promoter or enhancer.

In another embodiment of the invention, the polynucleotides encoding PAMP, or any fragment thereof, or antisense molecules, can be used for therapeutic purposes. In one aspect, antisense molecules to the PAMP encoding nucleic acid molecules can be used to block the transcription or translation of the mRNA. Specifically, cells can be transformed with sequences complementary to PAMP nucleic acid molecules. Such methods are well known in the art, and sense or antisense oligonucleotides or larger fragments, can be designed from various locations along the coding or control regions of sequences encoding PAMP. Thus, antisense molecules may be used to modulate PAMP activity, or to achieve regulation of gene function.

Expression vectors derived from retroviruses, adenovirus, adeno-associated virus (AAV), herpes or vaccinia viruses, or from various bacterial plasmids can be used for delivery of antisense nucleotide sequences to the prostate cell population. The viral vector selected should be able to infect the tumor cells and be safe to the host and cause minimal cell transformation. Retroviral vectors and adenoviruses offer an efficient, useful, and presently the best-characterized means of introducing and expressing foreign genes efficiently in mammalian cells. These vectors are well known in the art and have very broad host and cell type ranges, express genes stably and efficiently. Methods which are well known to those skilled in the art can be used to

construct such recombinant vectors and are described in Sambrook et al.(supra). Even in the absence of integration into the DNA, such vectors can continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression can last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system.

Ribozymes, enzymatic RNA molecules, can also be used to catalyze the specific cleavage of PAMP mRNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target PAMP RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within any potential RNA target are identified by scanning the PAMP RNA for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for secondary structural features which can render the oligonucleotide inoperable. The suitability of candidate targets can also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Antisense molecules and ribozymes of the invention can be prepared by any method known in the art for the synthesis of nucleic acid molecules.

In another enbodiment, the PAMP promoter and regulatory region can be used for constructing vectors for prostate cancer gene therapy. The promoter and regulatory region can be fused to a therapeutic gene for

prostate specific expression. This method can include the addition of one or more enhancer elements which amplify expression of the heterologous therapeutic gene without compromising tissue specificity. Methods for identifying the PAMP gene promoter and regulatory region are well known to those skilled in the art, for example, by selecting an appropriate primer from the 5' end of the coding sequence and isolating the promoter and regulatory region from genomic DNA.

Examples of therapeutic genes that are candidates for prostate gene therapy utilizing the PAMP promoter include suicide genes. The expression of suicide genes produces a protein or agent that directly or indirectly inhibits neoplastic prostate cell growth or promotes neoplastic prostate cell death. Suicide genes include genes encoding enzymes, oncogenes, tumor suppressor genes, genes encoding toxins, genes encoding cytokines, or a gene encoding oncostatin. The therapeutic gene can be expressed using the vectors described previously for antisense expression.

In accordance with another embodiment of the present invention, there are provided diagnostic systems, preferably in kit form, comprising at least one invention nucleic acid molecule or antibody in a suitable packaging material. The diagnostic kits containing nucleic acid molecules are derived from the PAMP-encoding nucleic acid molecules described herein. In one embodiment, for example, the diagnostic nucleic acid molecules are derived from SEQ ID NO:1 and can be oligonucleotides or probes of the invention. Invention diagnostic systems

are useful for assaying for the presence or absence of nucleic acid encoding PAMP in either genomic DNA or mRNA.

A suitable diagnostic system includes at least one invention nucleic acid molecule or antibody, as a separately packaged chemical reagent(s) in an amount sufficient for at least one assay. For a diagnostic kit containing a nucleic acid molecule of the invention, the kit will generally contain two or more nucleic acid molecules. When the diagnostic kit is to be used in PCR, the kit will contain at least two oligonucleotides that can serve as primers for PCR. Those of skill in the art can readily incorporate invention nucleic probes and/or primers or invention antibodies into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein. A kit containing a PAMP polypeptide-specific antibody can contain a reaction cocktail that provides the proper conditions for performing an assay, for example, an ELISA or other immunoassay, for determining the level of expression of a PAMP polypeptide in a sample, and can contain control samples that contain known amounts of a PAMP polypeptide and, if desired, a second antibody selective for the anti-PAMP antibody.

The contents of the kit of the invention, for example, PAMP nucleic acid molecules or antibodies, are contained in packaging material, preferably to provide a sterile, contaminant-free environment. In addition, the packaging material contains instructions indicating how the materials within the kit can be employed both to detect the presence or absence of a particular PAMP nucleic acid sequence or PAMP polypeptide or to diagnose

the presence of, or a predisposition for a condition associated with the presence or absence of PAMP such as prostate cancer. The instructions for use typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also included within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Isolation of full-length PAMP cDNA

This example describes the isolation of a full-length PAMP cDNA.

Rapid amplification of cDNA ends was performed as follows to isolate the full-length PAMP cDNA. Human prostate Marathon-ready cDNA (ClonTech; Palo Alto, CA) was used for RACE. cDNAs were also prepared from androgen stimulated LNCaP cells using the Marathon cDNA amplification kit (ClonTech) according to manufacturer's protocol and used for 5'-RACE. RACE primers were as follows: 9E1-594r (5'-TTTTGTATTTGGCATCTATTTTGCTGCGG-3'; SEQ ID NO:3), 9E1-669r (5'-TGCAGAATGGACATGGAGTCGTGG-3'; SEQ ID NO:4), 9E1-RC52 (5'-GCTGGGATGCTTGAGGGCTTGG-3'; SEQ ID NO: 5), 9E1-RC61 (5'-AAGGACCCTGCTGGGATGCTTGAG-3';

SEQ ID NO: 6). RACE reactions were performed according to the standard ClonTech protocol, and the resulting nucleic acids sequenced by standard methods.

As shown in Figure 1, the full-length PAMP cDNA contains 4485 nucleotides predicted to encode a protein of 1382 amino acids.

Using the TMPRED protein prediction program, the PAMP sequence shown in Figure 1 was predicted to contain at least 4 transmembrane domains. These domains are TM1 from amino acid 10 to amino acid 30 (21 residues); TM2 from amino acid 142 to amino acid 164 (23 residues); TM3 from amino acid 283 to amino acid 299 (17 residues); and TM4 from amino acid 430 to amino acid 452 (23 residues).

EXAMPLE II

Characterization of Androgen-Regulated and Prostate-Localized Expression of PAMP

This example confirms that expression of PAMP is androgen-regulated and that PAMP is highly expressed in normal and neoplastic prostate epithelium relative to other human tissues.

A. Androgen-regulated expression of PAMP

PAMP expression was studied in the prostate carcinoma cell line LNCaP. Androgen-regulated expression of PAMP was confirmed by Northern analysis using LNCaP RNA.

The prostate carcinoma cell line LNCaP was cultured in RPM1 1640 medium supplemented with 10% fetal calf serum (FCS) (Life Technologies, Germantown, MD). Twenty-four hours before androgen regulation experiments, LNCaP cells were transferred into RPMI 1640 media with 10% charcoal-stripped FCS (CS-FCS) (Life Technologies, Germantown, MD). Media was replaced with fresh CS-FCS media or CS-FCS supplemented with 1 nM of the synthetic androgen R1881 (NEN Life Science Products Inc.; Boston, MA). Cells were harvested for RNA isolation at 0, 4, 8, 12, 16, 24, 36 and 48 hour time points.

Northern analysis was performed with total RNA isolated from the cells at the indicated time points. Briefly, the LNCaP RNA was isolated using TRIzol (Life Technologies; Germantown, MD) according to the manufacturer's directions. A cDNA fragment containing nucleotides 3363 to 4485 of SEQ ID NO: 1 was labeled with [α-^{32}P] dCTP (Amersham) using rediprime II random primer labelling system (Amersham), and the probes purified with Sephadex G50 Nick column (Pharmacia). The RNA blot was prepared by fractionating 10 µg total RNAs on a 1.2% formaldehyde gel and blotting (Sambrook *et al.*, 1989). Northern hybridization was carried out in ExpressHyb™ hybridization solution (ClonTech). Northern blots were exposed to a phosphor screen (Molecular Dynamics), and the images were scanned into a computer with a Phosphorimager. Quantification was performed using ImageQuant program (Molecular Dynamics).

Phosphorimage quantitation of the Northern demonstrated an induction of PAMP expression that was maintained as up to 48 hours of androgen exposure with

synthetic androgen R1881 (Figure 2). PAMP expression could be detected after 4 hours of androgen supplementation and increased steadily through the 48-hour time point. These results demonstrate that expression of PAMP is induced by androgens.

B. Distribution of PAMP in fetal and adult human tissues

The distribution of PAMP transcripts in normal human tissues was also determined by Northern analysis performed as described above using a multiple tissue northern blot purchased from ClonTech and contained 2 µg of (poly)A+ RNA in each lane. A β-actin control probe was used to verify equivalent loading of RNA. Figure 3 shows northern analysis of PAMP expression in 16 adult human tissues. Four forms of PAMP were observed. In 16 adult tissues examined, the 2.0 and 3.2 kb PAMP transcripts were predominantly expressed in prostate tissues, with very low or no detectable expression levels in heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, testes, ovary, small intestine, colon, or peripheral leukocytes (see Figure 3). Two higher molecular weight forms of about 5.0 and 6.5 kb were also expressed in ovary and testis tissues.

Hybridization of PAMP to a multiple tissue expression (MTE) array containing 50 human tissues (ClonTech), both fetal and adult was performed as described above using the same probe. As shown in Figure 4, the only significant hybridization was to grid C7, which contains human prostate tissue.

Together with the results described above, these results demonstrate that PAMP is a prostate-specific gene that is induced by androgen.

EXAMPLE III

***In situ* hybridization analysis**

This example describes *in situ* hybridization analysis and shows that PAMP is expressed in epithelial cells in both normal prostate and prostate cancer cells.

A PCR product was generated from the 3' end of the ARSDR1 using primer 9E1insitu1 (5'-TGAAGAACTCTGCTTTCAGCTTCGC-3'; SEQ ID NO:7) and 9E1insitu2 (5'-AGGAAACAGCCTCCTGTGGAAAATG-3'; SEQ ID NO:8). The PCR product was cloned into vector PCRII-TOPO (Invitrogen) and subsequently linearized at either end with BamHI or EcoRV, and transcribed to generate sense and anti-sense digoxigenin-labeled probes. Both dig-dUTP labeled sense and anti-sense probe were prepared using a dig RNA labeling kit (Boehringer Mannheim) according to manufacturer's instructions.

In situ hybridization was performed on an automated instrument (Ventana Gen II, Ventana Medical Systems) essentially as follows. Formalin-fixed and paraffin-embedded prostate specimens were obtained from a previously surgical specimen tissue bank. Tissue sections (5 μm) were mounted onto Proma plus slides (VWR Scientific); deparaffinizeded in a 65°C oven for 2 hours; soaked three times (5 minutes each) in xylene; and rehydrated through graded alcohol with a final rinse in 2XSSC. Before hybridization, sections were digested with

proteinase I cocktail for 12 minutes at 37°C. Subsequently, 10 ng of either sense or anti-sense probe was applied in hybridization buffer. The probe was denatured at 65°C for 4 minutes and hybridization was carried out at 42°C for 360 minutes. The tissue sections were then rinsed with 2X, 1X and 0.1X SSC at 37°C. Hybridized probe was detected with mouse anti-dig antibody, and the signal amplified by consecutive application of biotin conjugated anti-mouse antibody and streptavidin-horseradish peroxidase. The *in situ* signal was visualized by DAB and counter-stained with hematoxylin.

The results of the *in situ* analysis are shown in Figure 5. As evidenced by the staining seen in panel A (antisense PAMP probe; prostate cancer tissue sample) and panel C (antisense PAMP probe; normal prostate gland tissue sample), PAMP is expressed in epithelial cells in both normal prostate and prostate cancer cells.

EXAMPLE IV

Chromosomal localization of PAMP gene

These results demonstrate that PAMP is localized to chromosome 4p15-4p11 between markers D4S756 and D4S174.

The medium-resolution Stanford G3 radiation hybrid panel was used to map the chromosomal localization of ARSDR1 with primers 9E1MapF (5'-ACGTGCAGATACAATGCTCCTGAG-3'; SEQ ID NO:9) and 9E1MapR (5'-CATGTCATCGTTTTGCCACCG-3'; SEQ ID NO:10). The PCR

conditions were 35 cycles of 94°C 30 seconds, 55°C 30 seconds and 72°C 30 seconds. The PCR patterns were entered into SHGC RH server (www.shgc.stanford.edu) for analysis.

The results obtained with the G3 panel localized PAMP to chromosome 4p15-4p11 between markers D4S756 and D4S174.

Throughout this application various publications have been referenced within parentheses. The disclosures of these publications are hereby incorporated by reference in their entirety into this application in order to more fully describe the state of the art to which this invention pertains.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.